Null Results in Brief

No Apparent Association between NAT1 and NAT2 Genotypes and Risk of Stomach Cancer

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Introduction

Intake of well-done meat, which contains heterocyclic amines, has been associated with stomach cancer in both experimental rodent and epidemiological studies (1–3). In addition, tobacco, which contains the heterocyclic amine (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine), has been consistently associated with increased risk of stomach cancer (4, 5). *N*-Acetyltransferase 1 and 2 enzymes encoded by *NAT1* and *NAT2* (6) activate the N-hydroxylated forms of heterocyclic amines to DNA adducts (7), which has given rise to the hypothesis that genetic variants associated with rapid activity may be associated with elevated risk of stomach cancer (8–10).

Both genes exhibit genetic polymorphisms in humans corresponding to slow and rapid acetylator phenotypes (11). Two previous studies (8, 9) have provided support for an increased risk of stomach cancer associated with the *NAT1*10* allele, and one (10) of three (8–10) published papers found an association between *NAT2* genotypes and stomach cancer risk. Here, we examined the relationship between *NAT1* and *NAT2* genotypes and stomach cancer.

Materials and Methods

Data were derived from a population-based case-control study of stomach cancer that was carried out in Warsaw, Poland, between 1994 and 1996, which has been described in detail (4). A 30-ml blood sample was collected from 304 cases and 433 controls. We have previously shown that demographic characteristics of this subgroup were similar to cases and controls without a blood sample (4). *NAT2* genotype was determined using a comprehensive PCR-RFLP assay (12) designed to distinguish among >25 *NAT2* alleles. *NAT1* genotype was determined by sequencing two parts of the *NAT1* gene (nucleotides 150–650 and 750-1150). Nucleotide sequence was determined after purification of the amplified PCR products with Qiaquick PCR Purification Kit (Qiagen, Valencia, CA) using the Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Electrophoresis and analysis of DNA sequence reactions were performed with an ABI 310 Genetic Analyzer. Genotype data were not available for 4–5% of subjects from whom a blood sample had been collected because of inadequate amount or quality of DNA.

ORs[2] and 95% CIs, which were used to estimate the association between stomach cancer and *NAT* genotypes and other risk factors, were calculated via unconditional logistic regression using SAS 6.12 (SAS Institute, Inc.). Previous papers from this study have shown associations between stomach cancer and cigarette smoking, a history of stomach cancer in a first-degree relative and *GSTT1* null genotype (4, 13, 14). ORs were adjusted for age, sex, education, pack-years of cigarette smoking, family history of stomach cancer, *GSTT1* genotype, years lived on a farm, and fruit intake. Gene-gene and gene-smoking multiplicative interactions were evaluated by the likelihood ratio test. We carried out additional subgroup analyses to explore associations previously reported (8, 9), using the same reference group and adjusting for the same risk factors.

Results

Subjects with one copy of the *NAT1*10* allele had a significantly decreased risk for stomach cancer, whereas the few subjects who were homozygous for this allele had a nonsignificant increased risk (Table 1*A*). There was no evidence of interaction with smoking and other risk factors, although there was low power to detect this (data not shown). To maximize the comparability of results from our study with the two previous reports (8, 9), we carried out analyses using the same reference group and combined subjects with one or two copies of *NAT1*10*. In contrast to the previous reports, we found no evidence of an increased risk and some support for a decreased risk (Table 1*B*).

There was no association between stomach cancer risk and *NAT2* genotype grouped into functional categories of slow, intermediate, and rapid activity (Table 1*A*) or with *NAT2* genotypes associated with the slow phenotype compared with *NAT2* combined rapid and intermediate activity genotypes (Table 1*B*). Also, there was no evidence of an interaction between *NAT2* genotype with tobacco smoking, *GSTT1* null genotype, or *NAT1*10* (data not shown).

Discussion

We found evidence of a protective effect of the *NAT1*10* allele among heterozygotes, but a gene-dosage effect was lacking in that risk was increased among the small numbers of subjects who were homozygotes for this allele. Boissy *et al.* (8) found a

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[2] The abbreviations used are: OR, odds ratio; CI, confidence interval.

Table 1 *NAT1* and *NAT2* genotypes and the risk of stomach cancer

A. *NAT* genotypes and the risk of stomach cancer in Warsaw, Poland

Genotypes	Cases	Controls	OR[a] (95% CI)	OR[b] (95% CI)
NAT1				
*4/*4	185	224	1.0	1.0
*10/any (except 10)	61	121	0.61 (0.42–0.88)	0.57 (0.39–0.85)
*10/*10	11	11	1.22 (0.52–2.89)	1.29 (0.51–3.28)
All others	220	278	1.0	1.0
*10/any (except 10)	61	121	0.64 (0.45–0.91)	0.59 (0.41–0.87)
*10/*10	11	11	1.30 (0.55–3.05)	1.38 (0.55–3.50)
NAT2				
Slow	160	223	1.0	1.0
Intermediate	108	158	0.96 (0.70–1.32)	1.12 (0.79–1.60)
Rapid	28	33	1.18 (0.68–2.03)	1.04 (0.56–1.94)

B. Summary of published studies

Study	Location	Study design	No. of cases[c]	No. of controls[c]	*NAT1* ref. group	OR[d] of *NAT1*10 (95% CI)	No. of cases[e]	No. of controls[e]	OR[f] of *NAT2* rapid/intermediate (95% CI)
Boissy *et al.* Ref. 8	United Kingdom	Hospital-based	80	98	*4/*4	2.6 (1.4–4.8)	91	112	1.3 (0.7–2.6)
Katoh *et al.* Ref. 9	Japan	Hospital-based	140	122	*4/*4 or *3/*3	1.4 (0.8–2.4)	140	122	0.6 (0.2–1.7)
Ladero *et al.* Ref. 10	Spain	Clinical-based					99	258	2.7 (1.6–4.7)
Lan *et al.*	Poland	Population-based	257	356	*4/*4	0.7 (0.5–0.9)	296	414	1.0 (0.7–1.3)
			257	357	*4/*4 or *3/*3	0.7 (0.5–0.9)			

[a] Adjusted for age and sex.
[b] Adjusted for age, sex, education, tobacco smoke, years lived on a farm, fruit intake, family history of stomach cancer, and *GSTT1* null genotype.
[c] Number of cases and controls for calculating the unadjusted OR for one or two *NAT1*10 alleles *versus NAT1*4/*4 or *3/*3.
[d] Unadjusted OR for one or two *NAT1*10 alleles *versus NAT1*4/*4 or *3/*3.
[e] Number of cases and controls for calculating the unadjusted OR of the combined rapid and intermediate *NAT2* acetylation alleles *versus* slow acetylation alleles.
[f] Unadjusted OR of the combined rapid and intermediate *NAT2* acetylation alleles *versus* slow acetylation alleles.

significant increased risk for the *NAT1*10* allele overall (Table 1*B*) and a particularly strong effect among the few cases (n = 41) with advanced stage (OR = 4.8, 95% CI = 2.3–10.1). In contrast, we found a decreased risk in the same subgroup (n = 146 cases; OR = 0.6, 95% CI = 0.4–0.9). Katoh *et al.* (9) found a nonsignificant increased risk of the *NAT1*10* allele overall (Table 1*B*) and a significant risk among heavy smokers (n = 59 cases; OR = 2.97, 95% CI = 1.23–7.14). In contrast, we found a nonsignificant decreased risk among heavy smokers (n = 101 cases; OR = 0.7, 95% CI = 0.4–1.2). On the basis of results from these two relatively small, hospital-based studies and our larger, population-based study, we believe that an association between the *NAT1*10* genotype and risk of stomach cancer is unlikely.

Before our study, three publications had evaluated the relationship between *NAT2* genotypes and stomach cancer risk (Table 1*B*). Two of them found no association with *NAT2* slow acetylation genotypes (8, 9) and one reported a significantly increased risk of the combined intermediate and rapid *NAT2* acetylation alleles *versus* the slow acetylation (10). The latter paper had only 99 cases, which included both incident and prevalent patients (10). Our paper, which was population-based and two to three times larger than the previous reports, found no association (Table 1*B*). Taken together, we believe that these studies suggest that there is no association between *NAT2* genotypes and the risk of stomach cancer.

The variation in study results could possibly be because of different levels of exposure to NAT1 and NAT2 substrates (Ref. 10; *e.g.*, heterocyclic amines) across study populations. However, we think this explanation is unlikely given that the main effects of *NAT1* and *NAT2* genotypes for stomach cancer risk, and the sample sizes in previous reports are not compelling in-and-of-themselves (8–10).

The strengths of our study include a population-based design and a relatively large sample size for the evaluation of main effects of these genotypes. This study had 80% power to detect an OR of 1.6 for subjects with one or two copies of *NAT1*10* compared with subjects with two copies of *NAT1*4* and 80% power to detect an OR of 1.5 for subjects with rapid/intermediate *versus* slow *NAT2* genotypes. Furthermore, the *NAT1* and *NAT2* genotypes were comprehensively analyzed by methods that detected essentially all potentially informative variants. This study does have several limitations. Despite its size, the number of subjects in the subgroup analyses was small, resulting in limited power. In addition, 27% of cases died before interview or phlebotomy, mostly because of advanced disease. If *NAT1* and *NAT2* genotypes are related to survival, then our results might not be generalizable to deceased cases, almost all of whom had advanced disease. However, analyses showed no evidence of an increased risk between *NAT1*or *NAT2* genotypes and tumor stage and included cases who had advanced disease but who were alive at the time of interview.

In summary, the weight of evidence from three previous studies (8–10) and our own suggests that it is unlikely that the *NAT1*10* or *NAT2* rapid/intermediate genotypes are related to stomach cancer risk.

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